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Effect of protease inhibitors on physiological and biochemical changes influencing keeping quality in gladiolus

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ABSTRACT

An experiment was conducted to evaluate 30 gladiolus varieties for their keeping quality in the field (field-life) and also under laboratory conditions during 2011-12. It was found that the varieties Big Time Supreme and Jackson Ville Gold recorded the maximum keeping quality in terms of field-life and vase-life (15.67, 13.67 and 13.67, 12.67 days, respectively). Minimum keeping quality was observed in the varieties Purple Flora (7.33 and 5.67 days) and White Friendship (8.00 and 6.33 days). The variety Big Time Supreme recorded highest floret diameter (10.30 cm) whereas minimum was recorded in Purple Flora (5.75 cm). The maximum vase water uptake was recorded in Jackson Ville Gold (63.00 ml) and Big Time Supreme (59.67 ml), whereas minimum was observed in Purple Flora (30.33 ml) followed by White Friendship (34.00 ml).Water uptake showed positive correlation with the vase-life. In another experiment, five protease inhibitors, *viz*., 5-sulfosalicylic acid, N-ethylemaleimide, aprotinin, leupeptin and E-64 were evaluated their effect on increasing the keeping quality of gladiolus cut spikes. It was found that 5-sulfosalicylic acid resulted in improvement of vase-life irrespective of the variety (12.33 day) over control (9.58 day) followed by N-ethylmaleimide and aprotinin (10.33 day each). The protease activity was found to decrease with the treatment of protease inhibitors and maximum reduction was recorded with the use of 5-sulfosalicylic acid. Similarly, the total soluble protein content was observed maximum in the same treatment.

Key words: Gladiolus, protease inhibitor, senescence, vase-life.

INTRODUCTION

Gladiolus is one of the important commercial cut flower both in the national as well as in the international market. The production of gladiolus cut spikes is increasing in India owing to increasing market demand and ease in its cultivation. Numbers of varieties are available in the market to choose from. However it is essential to consider the keeping quality, while selecting a variety. Longer keeping quality enables transport to longer distances, increases the storage life and saves the farmer from distress sale. Moreover, longer vase-life is one of the quality criteria consumers look for. An attempt was therefore made to evaluate the keeping quality of 30 gladiolus varieties. It has been reported that protease inhibitors play an important role in delaying the senescence and thereby increasing the vase-life (Arora and Singh, 2). Hence, five protease inhibitors, *viz.,* 5-sulfosalicylic acid, N-ethylemaleimide, aprotinin, leupeptin and E-64 were evaluated for their effect on increasing the keeping quality of gladiolus cut spikes.

MATERIALS AND METHODS

Healthy and uniform sized corms (4.0-5.0 cm) of 30 commercial gladiolus varieties procured from Directorate of Floricultural Research, New Delhi were used as experimental material. Planting of gladiolus corms was done at a spacing of 40 cm between the rows and 20 cm within the rows on 16th October in a randomized block design, recommended cultural practices were followed uniformly in all the plots. For both the experiments, the gladiolus spikes were harvested with sharp secateurs giving a slanting cut in the morning when the lower 1-2 florets showed colour. The spikes were kept in test tubes (25 mm dia. and 30 ml capacity) filled with distilled water after re-cutting in water. They were then observed for their keeping quality at room temperature in a factorial completely randomized design. The spikes were also observed for their field-life on the plant tagging them at the same stage. Three replications of each variety consisting of five spikes per replication were used. Five protease inhibitors, *viz*. 5-sulfosalicylic acid, leupeptin, E-64, N-ethylmaleimide, and aprotinin procured from M/s Sigma Chemicals, St. Louis USA, were evaluated for their effect in enhancing the vase-life of gladiolus cut spikes. Four gladiolus varieties, Big Time Supreme, Jackson Ville Gold, White Prosperity and Purple Flora (two each of high and low rate of senescence based on results of first experiment) were used in this experiment in Factorial Completely Randomized

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Design (FCRD) in the laboratory at room temperature. Each treatment was replicated thrice with five spikes in each replication.

Membrane stability index (MSI), total soluble protein and protease activity were studied at five stages of flower development, *viz*., bud stage (colour visible), half-open stage, fully-open stage, incipient senescent stage and finally senescence stage. Electrolyte leakage from petal tissue was measured during every stage of the flower development as MSI = (1- C_1/C_2) (Sairam *et al.*, 14).

Total protease activity of the petals was determined (Nieri *et al*., 12) using azocasein as a synthetic substrate with additional modifications. Sample activity was defined in arbitrary units with 1 unit equivalent to a change of 0.01 absorbance units per hour at 492 nm. Total soluble protein content was assayed by Bradford's method (5) based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, cause a visible colour change.

RESULTS AND DISCUSSION

It is well established that the post-harvest life and quality of cut flowers depends on pre-harvest, harvest and post-harvest factors. Genotypic or genetic constitution of a species is one of the pre-harvest factors influencing the keeping quality of cut flowers. The results of the present investigation, wherein different gladiolus varieties differed significantly also confirm this. Earlier, workers reported varietal variation in respect of keeping quality in gladiolus (Arora *et al*., 1).

Among the 30 gladiolus varieties evaluated, it was found that the varieties Big Time Supreme and Jackson Ville Gold recorded maximum keeping quality in terms of field-life and vase-life in the laboratory (15.67& 13.67 days and 13.67 &12.67 days, respectively) (Table 1). Minimum keeping quality was observed in the varieties Purple Flora (7.33 and 5.67 days) and White Friendship (8.00 and 6.33 days) in terms of field-life and vase-life, respectively.

Only two varieties, Big Time Supreme and Jackson Ville Gold were found in the very high vase-life category (Table 2). The varieties, Purple Flora, White Friendship and Peter Pears belonged to the low vase-life category. Most of them were in high vase-life category, *viz*., Eurovision, Ocilla, Red Beauty, Chemistry, Overture, Wigs Sensation, Priscilla, Fidelio, Trader Horn, Red Advance, Punjab Glance, Peasano, Yellow Stone, Alexander. The Great, Rose Supreme, White Prosperity, Pusa Suhagin and Punjab Elegance, while few were in medium category (Hunting Song, Snow Princess, Solist, Jessica, Novalux, Punjab Elegance and Mayur). Information on the vase-life of different gladiolus varieties enables a farmer to choose the variety with longer vase-life. Variation in vase-life offers ideal experimental system to investigate the mechanism of senescence. Accordingly, the varieties Big Time supreme & Jackson Ville Gold (recording higher vase-life) and Purple Flora & White Friendship (recording lower vase-life) were selected for the second experiment, which intended to evaluate the efficacy of protease inhibitors on the keeping quality of cut gladiolus spikes.

Floret size was highest in Big Time Supreme (10.30 cm dia.) and lowest in Purple Flora 5.75 cm dia.), which showed maximum and minimum vase-life, respectively. The trend of floret size, however, was not consistent with other varieties with respect to vase-life. Variation in floret size in different varieties was reported by several workers (De *et al*., 6) as it was supposed to be governed by the genetic constitution. It may be difficult to find a correlation between vase-life and floret size. Raju (13) reported improvement of vase-life with the use of thiol compounds in vase solutions without any improvement in flower size. Thus, flower size is more dependent on genotype and growing conditions.

Water uptake also showed similar trend. It was highest in the varieties Jackson Ville Gold (63.00 ml) and Big Time Supreme (59.67 ml), which recorded higher vase-life, and minimum in Purple Flora (30.33 ml) and White Friendship (34.00 ml). Similar results of higher water uptake for a better vase-life were reported by Arora *et al*. (3). The change in fresh weight did not show a consistent trend and hence it may not be possible to correlate change in fresh weight with the vase-life of the variety. Variety Hunting Song recorded the maximum change in fresh weight (4.63 g) followed by Peter Pears (3.76 g) (Table 3). Change in fresh weight in different varieties was found to be narrow since it did not show any particular trend. Yamane *et al*. (16), however, reported that with the commencement of senescence there was a rapid decline in fresh weight of gladiolus. Protease inhibitors were supposed to inhibit the activity of different proteases including cysteine proteases, which were reported to have significant role in degradation of proteins and promotion of senescence (Kant and Arora, 11). In the present investigation, all the protease inhibitors were found to enhance the vase-life of cut spikes of all the gladiolus varieties over control. 5-sulfosalicylic acid recorded the maximum improvement, which was also reported indicated earlier. The mode of action may be similar to that described by Ezhilmathi *et al*. (9). The varieties,

Table 1. Evaluation of gladiolus varieties for their keeping quality.

Variety	Field-life (days)	Vase-life (days)	Initial fresh wt. (g)	Final fresh wt. (g)	Change in fresh wt. (g)	2nd floret dia. (cm)	Water uptake (ml)
Peter Pears	8.67	6.67	61.93	58.17	3.76	9.25	49.33
Chemistry	10.00	8.33	42.50	40.87	1.63	8.70	45.40
Overture	9.33	8.00	54.97	54.40	0.57	9.85	40.17
Jackson Ville Gold	13.67	12.67	38.13	36.23	1.90	8.00	63.00
Hunting Song	10.00	7.67	38.60	33.97	4.63	8.00	44.33
Snow Princess	9.33	7.33	53.93	51.77	2.16	9.85	38.67
Wigs Sensation	12.00	8.00	50.07	49.13	0.94	8.10	36.83
Big Time Supreme	15.67	13.67	39.77	37.87	1.90	10.30	59.33
Priscilla	9.67	8.33	44.73	42.50	2.23	8.40	40.00
Fidelio	9.67	8.33	44.20	41.87	2.33	8.25	43.00
Purple Flora	7.33	5.67	28.13	26.07	2.06	5.75	29.67
Solist	8.67	7.33	44.97	42.60	2.37	7.90	44.67
Jessica	9.67	7.67	38.83	37.03	1.80	8.20	50.67
Novalux	9.67	7.33	50.10	48.13	1.97	7.35	42.33
Eurovision	10.67	9.33	47.03	44.80	2.23	8.70	45.33
Punjab Lemon Delight	9.67	7.67	40.03	37.93	2.10	6.85	42.33
Trader Horn	10.00	8.33	30.47	28.77	1.70	6.70	38.33
Red Advance	10.67	8.00	45.67	43.80	1.87	7.70	38.67
Punjab Glance	11.00	8.33	45.23	43.80	1.43	6.10	42.33
Peasano	10.33	8.00	42.57	40.87	1.70	7.00	39.33
White Friendship	8.00	6.33	34.40	32.87	1.53	8.10	34.00
Ocilla	11.00	9.33	45.67	44.57	1.10	8.00	43.33
Yellow Stone	10.67	8.33	48.30	48.23	0.07	8.75	46.33
Alexander The Great	9.67	8.00	39.67	38.97	0.70	8.40	46.67
Rose Supreme	10.67	8.33	50.47	48.20	2.27	9.25	50.00
Red Beauty	11.00	9.33	50.87	49.07	1.80	8.00	53.67
White Prosperity	10.67	8.33	55.20	53.07	2.13	9.00	44.67
Mayur	9.00	7.67	39.77	37.57	2.20	6.25	37.00
Pusa Suhagin	10.00	8.00	46.17	44.10	2.07	7.80	41.67
Punjab Elegance	9.33	8.00	48.83	46.53	2.30	9.25	48.67
$CD_{0.05}$	2.35	1.18	5.98	5.17		0.54	3.66

Table 2. Classification of gladiolus varieties based on vase-life.

Category	Vase-life (days)	Varieties
Very High	>10	Big Time Supreme and Jackson Ville Gold
High	8-10	Eurovision, Ocilla, Red Beauty, Chemistry, Overture, Wigs Sensation, Priscilla, Fidelio, Trader Horn, Red Advance, Punjab Glance, Peasano, Yellow Stone, Alexander The Great, Rose Supreme, White Prosperity, Pusa Suhagin and Punjab Elegance.
Medium	7-8	Hunting Song, Snow Princess, Solist, Jessica, Novalux, Punjab Lemon Delight and Mayur.
Low	<7	Purple Flora, White Friendship and Peter Pears.

Table 3. Effect of protease inhibitors on membrane stability index (%) in different stages of gladiolus flowers.

Treat (A)	Big Time Supreme (B1)					Jackson Ville Gold (B2)					White Friendship (B3)					Purple Flora (B4)				
stage	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
1	76.27	57.19	32.35	26.08	22.60	68.82	55.46	41.35	40.78	33.52	66.52	62.18	49.58	26.12	13.80	73.54	54.39	38.95	29.81	30.90
2	84.05	82.53	71.45	29.52	15.42	72.20	66.93	60.34	22.81	12.68	81.26	79.79	66.80	31.95	28.29	71.37	61.65	49.23	46.64	30.86
3	72.39	47.80	23.65	21.76	14.18	71.70	49.12	24.57	16.10	8.81	65.36	33.76	17.67	10.09	8.59	74.82	60.48	50.66	32.91	37.59
4	73.04	54.88	33.66	13.00	11.53	75.76	59.33	40.09	18.65	21.22	61.62	38.06	22.46	15.45	14.29	63.06	50.18	30.58	19.26	13.95
5	82.15	40.86	46.01	22.12	10.58	78.20	63.85	48.45	43.15	37.59	64.64	44.75	38.90	17.36	12.65	73.53	57.81	33.95	30.38	25.05
6	74.76	39.64	33.54	25.55	22.81	68.56	56.29	42.23	40.68	33.32	66.45	62.06	49.83	27.24	14.46	73.27	55.97	38.02	29.28	33.01
Mean	77.11	53.82	40.11	23.00	16.19	72.54	58.50	42.84	30.36	24.52	67.64	53.43	40.87	21.37	15.35	71.60	56.75	40.23	31.38	28.56
A × B	1	2	3	4	Mean	A × C	1	2	3	4	5	Mean		B × C	1	2	3	4	5	Mean
1	42.90	47.98	43.64	45.52	45.01	1	71.29	57.30	40.56	30.70	25.21	45.01		1	77.11	53.82	40.11	23.00	16.19	42.05
2	56.60	46.99	57.62	51.95	53.29	2	77.22	72.72	61.96	32.73	21.81	53.29		2	72.54	58.50	42.84	30.36	24.52	45.75
3	35.95	34.06	27.09	51.29	37.10	3	71.07	47.79	29.14	20.22	17.29	37.10		3	67.64	53.43	40.87	21.37	15.35	39.73
4	37.22	43.01	30.37	35.41	36.50	4	68.37	50.61	31.70	16.59	15.25	36.50		4	71.60	56.75	40.23	31.38	28.56	45.70
5	40.35	54.25	35.66	44.14	43.60	5	74.63	51.82	41.83	28.25	21.47	43.60		Mean	72.22	55.62	41.01	26.53	21.15	
6	39.26	48.22	44.01	45.91	44.35	6	70.76	53.49	40.91	30.69	25.90	44.35								
Mean	42.05	45.75	39.73	45.70		Mean	72.22	55.62	41.01	26.53	21.15									
$CD_{0.05}$	A (treatment)		0.36	A × B		0.72														
	B (variety)		0.30	A × C		0.81														
	C (stage)		0.33	B × C		0.66														

Treatments, 1 = Control, 2 = 5-sulfosalicylic acid, 3 = N-ethylmaleimide, 4 = Aprotenin, 5 = Leupeptin, 6 = E-64

Big Time Supreme and Jackson Ville Gold, which recorded maximum vase-life in the first experiment, have shown maximum response to the treatment with protease inhibitors, while the reverse was true with Purple Flora and White Friendship, which recorded the minimum vase-life.

The combination of Stage 1 and 5-sulfosalicylic acid in the variety Big Time Supreme resulted in maximum MSI (84.05%), whereas, minimum (8.59%) was recorded in Stage 5 of White Prosperity treated with N-ethylmaleimide. The interactions among different factors also showed similar trend except the interaction between treatment and variety wherein maximum (57.62) and minimum (27.09%) MSI was recorded in the same variety White Prosperity with the treatment of 5-sulfosalicylic acid and N ethylmaleimide, respectively (Table 3). Treatment with 5-sulfosalicylic acid resulted in maximum vase-life (12.33 days) (Table 4) compared to the control (9.58 days). The variety Big Time Supreme recorded maximum vase-life (15.00 days) and the variety Purple Flora showed lowest vase-life (7.44 days), which was at par with White Prosperity (7.72 days).

The action of 5-sulfosalicylic acid might be through maintaining the MSI and inhibiting the protease activity, which recorded the maximum MSI and minimum protease activity. Similar results were reported by Raju (13) in gladiolus cv. Pusa Jyotsna and Ezhilmathi *et al.* (9) in gladiolus cv. Snow Princess. The treatment with 5-sulfosalicylic acid recorded the highest soluble protein content and lowest protease activity in the variety Big Time Supreme, which was classified into very high vase-life category. These findings enable us to state that the 5-sulfosalicylic acid has strong protease inhibiting activity, which was evident from the higher protein content and both together helps in delaying senescence. Earlier, Arora (1) also reported similar results with 5-sulfosalicylic acid. During senescence, cellular organelles are dismantled and macro-molecules are degraded releasing nutrients for remobilization to other rapidly growing tissues in the plant. Protease action in tissues undergoing senescence provides the plant with transportable amino acids (*e.g.* asparagines) (Eason *et al*., 8).

In the present study, there was a gradual decrease in the total soluble protein content throughout the flower development (Fig. 2). The decrease was sharp and higher in magnitude from Stages 3 to 4 and 5. The results are in conformity with that of Arora and Singh (2) and Jones *et al*. (10). The protease inhibitors, 5-sulfosalicylic acid as well as N-ethylmaleimide significantly reduced the decline in protein content in all stages of flower development compared to control. These results are in agreement with Dwivedi (7) in gladiolus cv. Snow Princess with the use of nitric oxide. It appears that all those treatments, which can sustain the protein levels are helpful in delaying the senescence.

The protease activity in different stages of flower development showed a reverse trend to that of protein content (Fig. 1). Maximum protease activity was estimated in stage 5 (60.19 units g^{-1} FW.) and minimum was recorded at Stage 1 (4.04 units g^{-1} FW). There was considerable reduction protease activity with the treatment of 5-sulfosalicylic acid as well as N-ethylmaleimide. This explains the reduction in protein content brought out by these treatments compared to the control. There was a strong negative correlation between the protease activity and total soluble protein content indicating the role of proteases in the breakdown of macro-molecules during senescence as reported by Eason *et al.* (8). There is, however, growing evidence that specific proteases may also act as mediators of signal

Table 4. Effect of protease inhibitors on the vase-life of gladiolus cut spikes.

Variety	Big Time Supreme	Jackson Ville Gold	White Friendship	Purple Flora	Mean
Treatment					
Control	13.67	12.67	6.33	5.67	9.58
5-SSA	16.33	15.33	9.00	8.67	12.33
N-Ethylmaleimide	15.67	14.00	8.00	7.67	11.33
Aprotinin	15.33	14.00	7.67	8.33	11.33
Leupeptin	14.67	13.67	7.33	7.00	10.67
E-64	14.33	13.33	8.00	7.33	10.75
Mean	15.00	13.83	7.72	7.44	
$CD_{0.05}$	Treatment	0.71			
	Variety	0.58			
	T × V	NS			

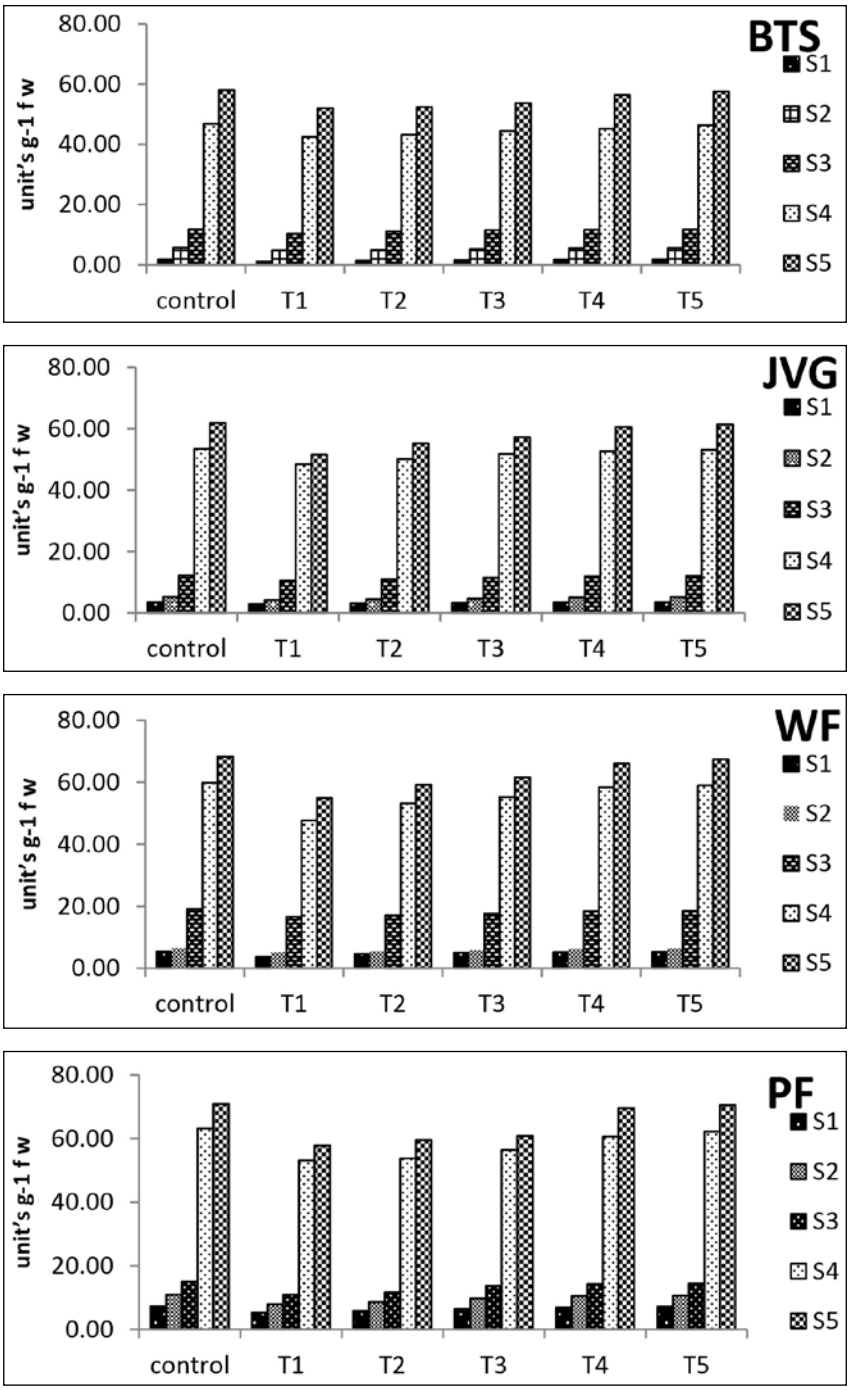

Fig. 1. Effect of protease inhibitors on protease activity (units g^{-1} FW) in different stages of gladiolus flowers. (BTS = Big Time Supreme, JVG = Jackson Ville Gold, WF = White Friendship, PF = Purple Flora), S1 = 5 (stages of flower development 1 to 5); T1-5 (control, 1 = 5-sulfosalicylic acid, 2 = N-ethylmaleimide, 3 = Aprotenin, 4 = Leupeptin, 5 = E-64).

transduction of effectors of programmed cell death during plant senescence (Beers *et al.*, 4). Through the *in vitro* use of specific protease inhibitors, researchers have attributed protease activity associated with flower senescence to cysteine proteases (Stephenson and Rubinstein, 15).

The varietal variation in respect of keeping quality may be explained by the contrasting results obtained

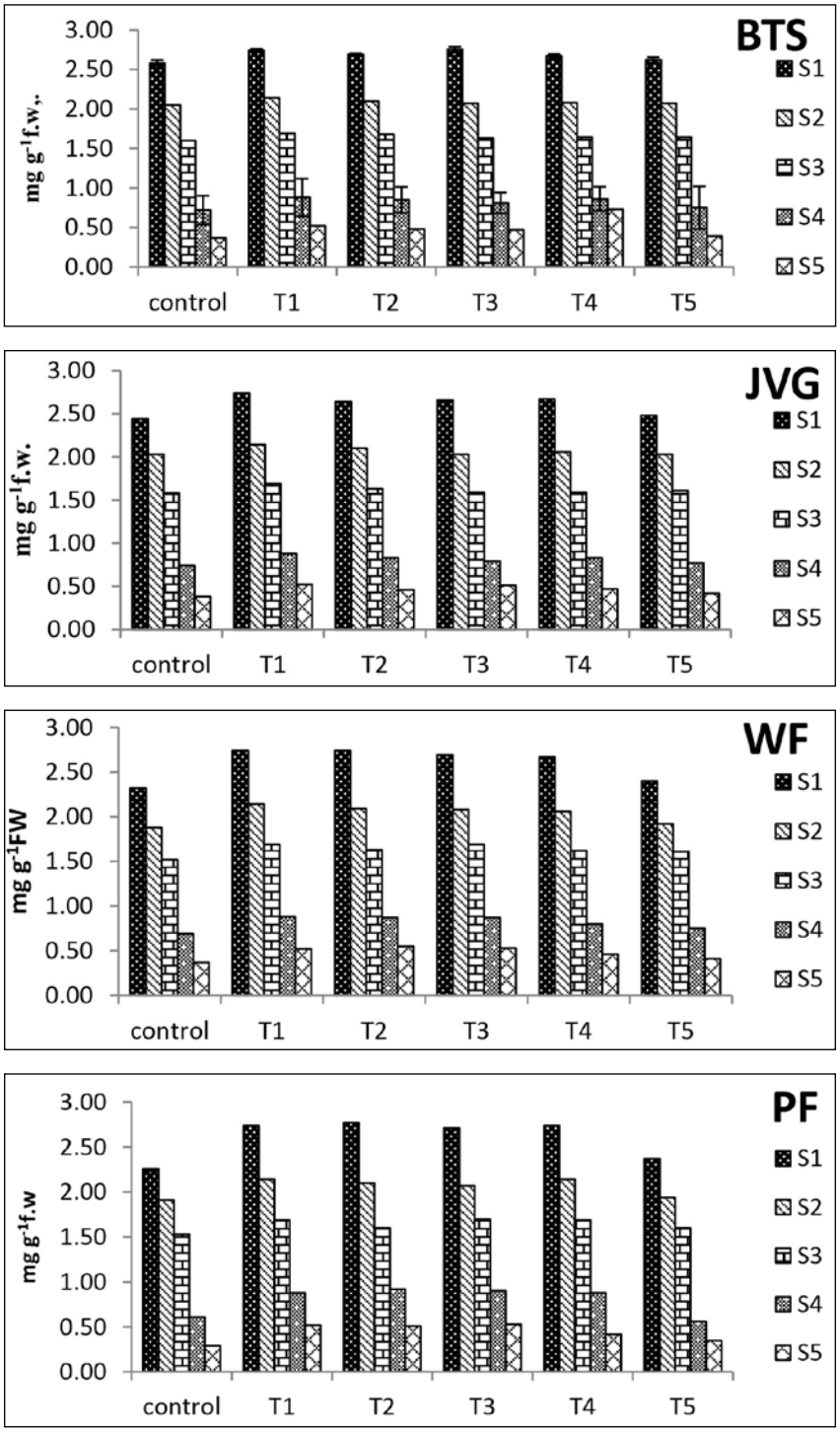

Fig. 2. Effect of protease inhibitors on total soluble protein (mg g^{-1} FW) content during different developmental stages of gladiolus flowers.

in terms of protease activity, protein content and MSI. The variety Big Time Supreme recorded significantly lowest mean protease activity (23.59 units g^{-1} FW.) and maximum was in Purple Flora (30.52 unit's g^{-1} FW.). It was minimum at stage 1 (1.20) with the treatment of 5-sulfosalicylic acid in Big Time Supreme and maximum at stage 5 (70.87) in control treatment of variety Purple Flora. The variety Big Time Supreme

showed the maximum total soluble protein content at stage 1 with the treatment of 5-sulfosalicylic acid (2.75 mg^{-1} FW) (Table 6) and N-ethylmaleimide (2.74 mg^{-1} FW), whereas, minimum (0.29 mg^{-1} FW) was recorded in the variety Purple Flora in stage 5 treated with distilled water. The combination of stage 1 and 5-sulfosalicylic acid in the variety Big Time Supreme resulted in maximum MSI (84.05%), whereas, minimum (8.59%) was recorded in stage 5 of White Friendship treated with N-ethylmaleimide.

From the negative correlation between soluble protein content and the activity of proteases it can be concluded that maintenance of protein resources simultaneously keeping the activity of proteases to a minimum would be helpful in delaying senescence in gladiolus cut spikes. This makes the gladiolus flowers to serve as good model system for all those flowers in which protease mediated senescence is dominant or those flowers, which are ethylene insensitive.

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